DATA EVALUATION RECORD LIFE-CYCLE SEDIMENT *Chironomus tentans* TOXICITY TEST (FOLLOWING EPA TEST METHOD 100.5)

1. **CHEMICAL**: Bifenthrin PC Code No.: 128825 **2. TEST MATERIAL**: Bifenthrin (tech.) Purity: 93.6% 3. CITATION: Authors: Picard, C.R. Title: Life-Cycle Toxicity Test Exposing Midges (Chironomus dilutus) to Bifenthrin Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. Study Completion Date: December 12, 2013 **Laboratory**: Smithers Viscient 790 Main Street Wareham, MA 02571-1037 Sponsor: Consumer Specialty Products Association, Inc. for the Bifenthrin Task Force Steering Committee/Joint Venture 1667 K Street, NW, Suite 300 Washington, DC 20006 Laboratory Report ID: 14011.6125 MRID No.: 49277502 DP Barcode: D418946 4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation Signature: Christie E. Padova **Date:** 08/04/14 **APPROVED BY:** John Marton, Ph.D., Environment Scientist, CDM Smith Signature: **Date:** 06/01/15 5. APPROVED BY: **Signature:** Date: 6. STUDY PARAMETERS **Scientific Name of Test Organism:** Chironomus dilutus Age of Test Organism: First instar larvae, 4 days old **Definitive Test Duration:** 63 days Intermittent flow-through **Study Method: Type of Concentrations:** TWA bulk and OC-normalized

sediment and TWA porewater

concentrations

7. **CONCLUSIONS**:

Results Synopsis:

TWA SEDIMENT

NOAEC: 46 μg ai/kg LOAEC: 93 μg ai/kg

TWA SEDIMENT, OC-NORMALIZED

NOAEC: 1.7 μg ai/g OC LOAEC: 3.3 μg ai/g OC

TWA SEDIMENT, POREWATER

NOAEC: 0.41 µg ai/L LOAEC: 0.69 µg ai/L

Endpoint(s) Affected: midge larval growth (AFDW, Day20)

Most Sensitive Endpoint(s): midge larval growth (AFDW, Day 20)

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Unacceptable

B. Rationale: This study followed methods described in the "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates", 2nd Edition, Test Method 100.5: "Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chrionomus tentans*." March 2000 (EPA 600/R-99/064).

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

This study does not fulfill any current U.S. EPA guideline requirement. Deviations from U.S. EPA Test Method 100.5 included:

- The larvae used to initiate the test were 4 days old, whereas guidance specifies larvae should be newly-hatched, <24-hours old.
- The temperature range of the overlying water exceeded recommendations (23 ± 1 °C) on Days 5 through 11, 14 through 17, 28, 29 and 55, with a minimum recorded value of 19.5°C and a maximum measurement of 25.6°C.
- Variability (CV) in overlying water ammonia (NH₃-N) concentrations ranged from 80.9 to 98.7%, exceeding the recommended 50%.
- The number of eggs per egg mass averaged only 776 and 742 for the negative and solvent control groups, respectively, whereas the mean number of control eggs/egg case should be ≥ 800 .

10. MATERIALS AND METHODS

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species Chironomus dilutus (formerly C. tentans)	Chironomus dilutus
Life Stage <24-hr old larvae	4-day old larvae
Source Collect a min. of 6 to 8 egg cases.	Egg masses were obtained from the laboratory's culture facility.
All organisms from the same source?	Yes

Guideline Criteria	Reported Information
Culture Conditions Transfer all egg cases to a crystallizing dish containing control water. Discard larvae that have already left the cases. Test organisms must be cultured and tested at 23°C, ideally in the same water used for testing.	Egg masses were placed in 270-mL crystallizing dishes containing approximately 200 mL of laboratory well water. The egg masses were maintained at 23 ± 1°C and observed daily until hatch. Hatched larvae were transferred to a shallow glass bowl containing 1 L of culture water and 2.5 mL of <i>Ankistrodesmus falcatus</i> (at 4 x 10 ⁷ cells/mL) to serve as a substrate. Larvae were reared for 4 days under static conditions with gentle oil-free aeration. During rearing, the temperature ranged from 22 to 24°C and the dissolved oxygen content ranged from 7.8 to 8.2 mg/L.
Feeding	During the short-term rearing, midge larvae were fed a finely-ground flaked fish food suspension (4.0 mg/mL).

B. Test System

Guideline Criteria	Reported Information
Dilution water (overlying water) Culture water, well water, surface water, site water, or reconstituted water	Laboratory well water characterized as having a total hardness and total alkalinity of 38 to 52 and 20 to 22 mg/L as CaCO ₃ , respectively, a pH range of 6.9 to 7.8, and a conductivity range of 210 to 350 μ S/cm.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 23 ± 1°C	Daily: 20 to 25°C Continuous: 20 to 26°C
<u>рН</u>	6.7 to 7.4

Guideline Criteria	Reported Information
Dissolved Oxygen >2.5 mg/L	3.2 to 8.2 mg/L
Ammonia Should not vary more than 50%.	0.15 to 1.6 mg/L as N
	Coefficients of variation exceeded 80% in all treatment groups.
Hardness Should not vary more than 50%.	32 to 60 mg/L as CaCO ₃
Alkalinity Should not vary more than 50%.	16 to 28 mg/L as CaCO ₃
Conductivity	250 to 350 μS/cm
Test Sediment Natural or formulated sediment	Artificial sediment (Batch No. 042413A) was prepared according to OECD Guideline No. 218 (2004) by mixing the following components (on a dry weight basis): 2.4 kg sphagnum peat, 8.0 kg kaolin clay and 29.6 kg fine sand (6.0, 20, and 74%, respectively). While blending (all the sediment components) using a large-scale laboratory mixer, an additional 8 L of laboratory well water was also added. Prior to blending, the peat was pre-soaked in
	dilution water for 1 week. During this time, the peat was amended with 110 g of powdered CaCO ₃ to increase the pH from 3.4 to 5.5.

Guideline Criteria	Reported Information
Sediment Characterization pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content should be reported	Particle distribution – 78% sand, 5% silt, 17% clay TOC – 2.8% Percent solids – 60.06% pH – 6.3 Ammonia concentration of pore water on Day 0 in the control group – 4.3 mg/L (as N)
Test Material	Bifenthrin technical IUPAC name: 2-methylbiphenyl-3-ylmethyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3- trifluoroprop-1-enyl]-2,2- dimethylcyclopropanecarboxylate CAS name: (2-methyl[1,1'-biphenyl]-3- yl)methyl (1R,3R)-rel-3-[(1Z)-2-chloro- 3,3,3-trifluoro-1-propen-1-yl]-2,2- dimethylcyclopropanecarboxylate CAS No.: 82657-04-3 Description: liquid Lot no.: PL09-0251 Purity: 93.6% Storage: room temperature, dark A 150 μg/mL primary stock solution was prepared by bringing 0.01603 g of test substance (0.0150 g bifenthrin) to 100 mL with acetone. The primary stock was clear and colorless with no visible un-dissolved test substance. Five individual dosing stock solutions were prepared (at 1.55 to 24.6 μg/mL) by diluting the appropriate volume of primary stock into 25 mL acetone. Dosing stock solutions were clear and colorless with no visible un-dissolved test substance.

Guideline Criteria	Reported Information
Solvents	Acetone, 10 mL/2.4524 kg dw
	The acetone was allowed to completely evaporate during the mixing procedure.
	Both solvent control and negative control groups were included in the study.
Sediment Spiking	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand, mixed into the sand with a metal spatula for <i>ca.</i> 2 minutes, and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 4.0 kg of wet sediment (total of 2.4524 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at <i>ca.</i> 15 rpm at room temperature. The range of nominal concentrations (6.3 to
	100 μg/kg) was based upon the results of a preliminary range-finding study and in consultation with the Sponsor.
Sediment Conditioning	The jars containing treated sediment were stored upright at 2 to 8°C for a 14-day equilibration period.
	Once a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours at room temperature to ensure the sediment was homogeneous.

Guideline Criteria	Reported Information
Test Vessels 300 mL high-form lipless glass beakers containing 100 mL of sediment and 175 ml of overlying water	300-mL glass vessels, each with two slots cut on the top edge covered with 40-mesh Nitex® screen for drainage.
	Each vessel contained 100 mL (ca. 4.0-cm layer) of sediment (equivalent to 93.1 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approx. 275 mL.
	On Day 13, emergence traps were placed over the test vessels to trap emergent flies for the remainder of the test. The emergence traps were 3.5-cm tall Plexiglass® tubes (6-cm id) covered with wide-mesh Nitex® screen.
Reproductive/oviposit chambers	Plexiglass tubes (3.5-cm length, 6-cm id) covered on the top with wide-mesh Nitex® screen and placed within a 100 x 20-mm Petri dish. With midge addition, 50 mL of laboratory well water was added to the Petri dish.
Type of Dilution System Continuous or intermittent	Intermittent flow-through
Flow Rate 2 volume additions/day	Initially, 2 volume additions/day – increased early on in the exposure [sic] to 4 volume additions/day.
Aeration None, unless DO in overlying water drops <2.5 mg/L	None reported
Photoperiod 16 hours light, 8 hours dark using wide- spectrum fluorescent lights; intensity of 100 to 1000 lux	16-hour light/8-hour dark photoperiod using fluorescent bulbs at an intensity range of 330 to 690 lux.

Guideline Criteria	Reported Information
<u>Feeding</u>	Midge larvae were fed a finely-ground flaked fish food suspension (4.0 mg/mL). During exposure, the food was introduced at a rate of 1.5 mL of flaked fish food suspension per test vessel per day.

C. Test Design

Guideline Criteria	Reported Information
Duration About 50 to 65 days; each treatment is ended separately when no additional emergence occurs for 7 consecutive days. When no emergence is recorded from a treatment, termination of that level is based on the control sediment using the 7-day criterion.	63 days
Sediment Into Test Chambers One day prior (Day -1) to start of test: each sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	Test systems were established on Day -1. Overlying water was gently added using a turbulence reducer, and each vessel was placed under the renewal system. Additional test systems were established on Day 9 for auxiliary male production.

Guideline Criteria	Reported Information
Renewal of Overlying Water Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test systems were observed daily for dead organisms (larvae or pupae) on the sediment surface, organism behavior (e.g., sublethal effects), and characteristics of test solutions. Also daily beginning on Day 19, the number of emerged midges was recorded (male and female), and once established, reproductive/oviposit chambers were checked daily for dead adults and egg masses.

Guideline Criteria	Reported Information
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.	 Preliminary toxicity assessment 53-day exposure at nominal levels of 0 (negative and solvent controls), 0.29, 1.2, 4.7, 19 and 75 mg/kg Twelve, <24-hour old larvae per replicate, with eight replicates per level established on Day 0 plus two replicates per level initiated on Day 10 (auxiliary male production) Day-20 survival averaged 89, 69, 72, 72, 78, 83 and 39%, respectively; difference was significant (p<0.05) at the 75 mg/kg level (excluded from further statistical analysis). No further effects observed for the following endpoints (up through 19 mg/kg): Day-20 AFDW (1.05 to 1.57 mg), percent emergence (55 to 75%), male emergence rate (0.0420 to 0.0479), female emergence rate (0.0420 to 0.0418), male days to death (2.0 to 2.8), female days to death (2.2 to 2.9), egg masses per mated female (0.65 to 1.0), eggs per egg mass (801 to 1093), number of eggs per mated female (648 to 1050), egg hatchability (76 to 94%), and days to oviposition (1.0 to 1.3).
Nominal Concentrations of Definitive Test	0 (negative and solvent controls), 6.3, 13, 25, 50 and 100 μg/kg dw sediment
Number of Test Organisms 12 organisms per test chamber are recommended; 16 replicates per treatment should be used (twelve at Day -1 and four for auxiliary males on Day 10)	12 organisms per test chamber 16 biological replicates per level

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning, on Day 20, and at the end of the test. Conductivity should also be determined weekly.	For all levels, total hardness, alkalinity, conductivity and ammonia concentrations were measured in a composite sample of the overlying water on Days 0, 10, 20, and 63.
DO and pH should be measured at the beginning of the test and at least three times per week thereafter. Temperature should be measured daily in one test chamber from each treatment. The temperature of the water bath should be continuously monitored.	DO, temperature, and pH were measured in the overlying water of each replicate vessel on Days 0, 10, 20, and 63. On the remaining days, DO and temperature were measured in one alternating replicate from each level, and the temperature was continuously monitored in an auxiliary vessel in the water bath.
Chemical Analysis	Concentrations of bifenthrin were determined in sediment and pore water from surrogate test vessels collected on Days 0, 20, and 63. The overlying water was decanted and discarded, and the sediment was centrifuged at <i>ca.</i> 10,000 <i>g</i> for 30 minutes to isolate the pore water. Sediment and pore water samples were analyzed using liquid chromatography with mass spectrometry (LC/MS/MS) and gas chromatography with mass selective detection (GC/MSD), respectively, based on

11. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: routine water, sediment and food contaminant screening analyses for pesticides, PCBs, and toxic metals. These analyses, however, were performed using certified laboratories and standard validated methods.
Control Criteria Was control survival at least 70% and dry weight at least 0.6 mg/organism (0.48 mg/organisms AFDW) on Day 20?	Negative control: 96% and 0.89 mg/larva Solvent control: 100% and 0.88 mg/larva
Was control emergence ≥50%? Was the mean number of control eggs/egg case ≥800 and was the percent hatch ≥80%?	Negative control: 71% Solvent control: 71% Negative control: 776 eggs/mass and 88% Solvent control: 742 eggs/case and 95%

Guideline Criteria	Reported Information
Percent Recovery of Chemical	Procedural recoveries (from QC samples) conducted concurrently with sample analysis:
	Sediment: Recovery range of 71.5 to 116% (n=7) (excludes two outliers of 67.3 and 128%) LOQ sediment = 0.39 to 0.46 μg ai/kg
	Reviewer-calculated time-weighted average bulk sediment concentrations were 5.3, 12, 23, 46, and 93 µg ai/kg, which corresponded to 0.19, 0.43, 0.81, 1.7, and 3.3 µg ai/g OC.
	Aqueous: Recovery range of 71.1 to 111% (n=7) (exclues two outliers of 455 and 138%) LOQ water = 0.0074 to 0.0080 μg ai/L
	Reviewer-calculated time-weighted average porewater concentrations were 0.060, 0.10, 0.19, 0.41, and 0.69 µg ai/L.
Data Endpoints	Day 20: - Survival - Ash-free dry weight (AFDW) Day 63: - Percent emergence - Male and female emergence rates - Male and female days to death (for mated midges) - Eggs masses per mated female - Eggs per egg mass - Eggs per mated female - Percent hatch - Days to oviposition
Raw data included?	Yes, sufficient.

Effects Data:

20-Day Endpoints:

Following 20 days, survival averaged 96 and 100% in the negative and solvent control groups, respectively, and ranged from 94 to 100% for all treatment levels, with no statistically-significant differences indicated. The NOAEC and LOAEC for 20-Day survival were 89 and >89 μ g ai/kg, respectively, and the observed 20-Day LC₅₀ was >89 μ g ai/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.8%), the NOAEC, LOAEC, and LC₅₀ for 20-Day survival were 3.2, >3.2, and >3.2 μ g ai/g OC, respectively.

Mean ash-free dry weights (AFDW) on Day 20 were 0.89 and 0.88 mg per larvae for the negative and solvent control levels, respectively, and 0.88, 0.91, 0.86, 0.75 and 0.68 mg per larvae for the mean-measured 5.2, 12, 22, 45 and 89 μg ai/kg treatment levels, respectively. The difference was statistically-significant compared to the negative control at the 89 μg ai/kg treatment level (p<0.05; Dunnett's Multiple Comparison Test). No concentration resulted in \geq 50% reduction in AFDW compared to the negative control, and therefore the NOAEC, LOAEC and observed EC₅₀ for 20-Day AFDW were 45, 89, and >89 μg ai/kg, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.8%), the NOAEC, LOAEC, and EC₅₀ for 20-Day AFDW were 1.6, 3.2 and >3.2 μg ai/g OC.

	nc., Sediment kg dw)	Day 20	
Nominal Sediment, μg ai/kg	Mean-measured Sediment, μg ai/kg	Survival (% ± SD)	AFDW (mg/larva ± SD)
Ctrl	<loq<sup>(a)</loq<sup>	96 ± 5	0.89 ± 0.13
Sol. Ctrl	<loq<sup>(a)</loq<sup>	100 ± 0	0.88 ± 0.14
6.3	5.2	100 ± 0	0.88 ± 0.12
13	12	96 ± 8	0.91 ± 0.040
25	22	96 ± 5	0.86 ± 0.14
50	45	98 ± 4	0.75 ± 0.078
100	89	94 ± 8	$0.68 \pm 0.14*$

^{*} Statistically-significant compared to the negative control (Dunnett Multiple Comparison Test, p<0.05).

⁽a) LOQ = 0.39 to $0.46 \mu g$ ai/kg.

63-Day Endpoints:

No treatment-related effects on emergence or time to death were observed at any level, with no statistically-significant differences from the negative control indicated for percent emergence, male emergence rate, male days to death or female days to death. There was a statistically-significant reduction at the 45 μ g ai/kg level compared to the negative control (p<0.05; Bonferroni's Adjusted t-Test) regarding the mean female emergence rate; however, the reduction was not concentration-responsive and not considered to be toxicant-related. For all emergence and time to death endpoints, the NOAEC and LOAEC were 89 and >89 μ g ai/kg, respectively, and the observed EC₅₀ was >89 μ g ai/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 2.8%), the NOAEC, LOAEC, and EC₅₀ for all emergence and time to death endpoints were 3.2, >3.2, and >3.2 μ g ai/g OC, respectively.

Toxicant Cor (μg ai/	nc., Sediment kg dw)			Day 63		
Nominal	Mean- measured	Percent Emergence ± SD	Male Emergence Rate ± SD	Female Emergence Rate ± SD	Male Days to Death ± SD	Female Days to Death ± SD
Ctrl	<loq<sup>(a)</loq<sup>	71 ± 13	0.0293 ± 0.0045	0.0248 ± 0.0045	4.6 ± 1.2	3.9 ± 0.50
Sol. Ctrl	<loq<sup>(a)</loq<sup>	71 ± 14	0.0315 ± 0.0059	0.0243 ± 0.0039	3.7 ± 0.76	3.9 ± 0.43
6.3	5.2	68 ± 7	0.0280 ± 0.0031	0.0256 ± 0.0031	3.8 ± 0.72	3.9 ± 0.55
13	12	76 ± 7	0.0310 ± 0.0036	0.0258 ± 0.0028	3.6 ± 0.87	3.7 ± 0.52
25	22	68 ± 17	0.0295 ± 0.0046	0.0244 ± 0.0032	3.5 ± 0.95	4.0 ± 1.2
50	45	58 ± 24	0.0275 ± 0.0044	$0.0206 \pm 0.0032*$	3.2 ± 1.6	3.8 ± 0.68
100	89	72 ± 10	0.0283 ± 0.0055	0.0241 ± 0.0023	4.0 ± 1.5	4.3 ± 0.64

^{*} Statistically-significant compared to the negative control (p<0.05). $^{(a)}$ LOQ = 0.39 to 0.46 µg ai/kg.

No treatment-related effects on reproduction were observed at any level, with no statistically-significant differences from the negative control indicated for the number of egg masses per mated female, the number of eggs per mated female, percent hatch or days to oviposition. There was a statistically-significant reduction compared to the negative control regarding the mean eggs per egg mass at the 45 μ g ai/kg level (p<0.05; Wilcoxon's Test with Bonferroni's Adjustment); however, the reduction was not concentration-responsive and not considered to be toxicant-related. For all reproductive endpoints, the NOAEC and LOAEC were 89 and >89 μ g ai/kg, respectively, and the EC₅₀ was >89 μ g ai/kg. When normalized for organic carbon (OC) content of the sediment (i.e., 2.8%), the NOAEC and LOAEC were 3.2 and >3.2 μ g ai/g OC, and the EC₅₀ was >3.2 μ g ai/g OC.

Toxicant Conc., Sediment (μg ai/kg dw)		Day 63				
Nominal	Mean- measured	Egg Masses per Mated Female ± SD	Eggs per Egg Mass ± SD	Eggs per Mated Female ± SD	Percent Hatch ± SD	Days to Oviposition ± SD
Ctrl	<loq<sup>(a)</loq<sup>	0.87 ± 0.16	776 ± 139	664 ± 123	88 ± 8	1.3 ± 0.31
Sol. Ctrl	<loq<sup>(a)</loq<sup>	0.80 ± 0.28	742 ± 122	620 ± 278	95 ± 4	1.5 ± 0.34
6.3	5.2	0.86 ± 0.18	746 ± 226	661 ± 285	90 ± 8	1.9 ± 1.7
13	12	0.98 ± 0.053	717 ± 108	705 ± 128	95 ± 2	1.4 ± 0.29
25	22	0.57 ± 0.44	803 ± 181	476 ± 413	82 ± 10	1.4 ± 0.42
50	45	0.93 ± 0.14	538 ± 139*	504 ± 171	86 ± 7	1.5 ± 0.47
100	89	0.62 ± 0.21	887 ± 217	540 ± 196	86 ± 18	1.1 ± 0.20

^{*} Statistically-significant compared to the negative control (p<0.05).

Analytical:

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for bifenthrin. Recoveries in the stock solutions ranged from 82 to 94% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 160 to 320% (see Reviewer's Comments).

⁽a) LOQ = 0.39 to $0.46 \mu g$ ai/kg.

During testing, concentrations of bifenthrin were determined in sediment and pore water on Days 0, 20, and 63. Results demonstrated that the majority of the test substance applied to the sediment remained associated with the sediment throughout the exposure.

For the nominal 6.3, 13, 25, 50 and 100 μ g/kg levels, sediment concentrations measured 4.7, 10, 19, 40 and 74 μ g ai/kg, respectively, on Day 0; 5.4, 12, 23, 47 and 99 μ g ai/kg, respectively, on Day 20; and 5.5, 13, 24, 48 and 93 μ g ai/kg, respectively, on Day 63. Calculated mean-measured concentrations represented 83 to 92% of nominal sediment levels.

For the nominal 6.3, 13, 25, 50 and 100 μ g/kg levels, pore water concentrations measured 0.042, 0.12, 0.21, 0.49 and 0.89 μ g ai/L, respectively, on Day 0; 0.082, 0.11, 0.22, 0.48 and 0.84 μ g ai/L, respectively, on Day 20; and 0.037, 0.081, 0.14, 0.26 and 0.39 μ g ai/L, respectively, on Day 28.

B. Statistical Results (From Study Report)

Endpoints that were statistically-analyzed included larval survival and growth (ash-free dry weight; AFDW) on Day 20; and percent emergence, emergence rate (gender-specific), time to death (gender-specific), eggs masses per mated female, eggs per egg mass, eggs per mated female, percent hatch, and days to oviposition (on Day 63). Analyses were performed with CETISTM (version 1.8, 2013) statistical software. Percent survival, percent emergence and percent hatch data were transformed (e.g., arcsine square-root percentage) prior to analysis. Results were provided in terms of mean-measured sediment concentrations and OC-normalized sediment concentrations (see Reviewer's Comments section).

An Equal Variance Two-Sample t-Test, Unequal Variance Two-Sample t-Test, or Wilcoxon's Rank Sum Two-Sample Test was used to compare the performance of the negative control and solvent control data. Regardless of the results of the comparisons (not reported), the treatment groups were compared to the negative control data to determine potential treatment-related effects.

For all endpoints, the data were tested for normality using the Shapiro-Wilks' Test, and for homogeneity of variance using a Modified Levene's Test or Bartlett's Equality of Variance Test. Time to oviposition data did not meet the assumption for normality, and the number of eggs masses per female data did not meet the assumption for homogeneity of variance. Thus, these endpoints were analyzed using Wilcoxon's Test with Bonferroni's Adjustment. All remaining endpoints met both assumptions and were assessed using Bonferroni's Adjusted t-Test or Dunnett's Multiple Comparison Test. NOAEC and LOAEC values were assigned based on significance. All statistical analyses were conducted at the 95% level of

certainty except in the case of the qualification tests (i.e., Shapiro-Wilks', Modified Levene's, and Bartlett's Tests), in which a 99% level of certainty was applied.

During the study, no concentration tested resulted in \geq 50% mortality or a \geq 50% reduction in any endpoint; therefore, the LC/EC₅₀ values were empirically estimated to be greater than the highest mean-measured sediment concentration tested.

Endpoint	Methods	Mean-measured Sediment, μg ai/kg	OC-normalized Sediment, μg ai/g OC
20-Day survival	Dunnett's Multiple Comparison Test	NOAEC: 89 LOAEC: >89 LC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 LC ₅₀ : >3.2 95% C.I.: N/A
20-Day AFDW	Dunnett's Multiple Comparison Test	NOAEC: 45 LOAEC: 89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 1.6 LOAEC: 3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Percent emergence	Dunnett's Multiple Comparison Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Male emergence rate	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Female emergence rate	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Male days to death	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Female days to death	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, μg ai/g OC
Egg masses/mated female	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Eggs/egg mass	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Eggs/mated female	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Percent hatch	Bonferroni's Adjusted t- Test	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A
Days to oviposition	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 89 LOAEC: >89 EC ₅₀ : >89 95% C.I.: N/A	NOAEC: 3.2 LOAEC: >3.2 EC ₅₀ : >3.2 95% C.I.: N/A

Endpoint(s) Affected: midge larval growth (AFDW, Day20)

Most Sensitive Endpoint(s): midge larval growth (AFDW, Day 20)

12. <u>VERIFICATION OF STATISTICAL RESULTS</u>

Statistical Method: The reviewer analyzed Day 20 growth and survival and Day 63 emergence and reproductive endpoints using CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Negative and solvent control data were compared for each endpoint using a two-sample t-test assuming equal variance. No significant differences were detected between the controls for any endpoint. All subsequent analyses were conducted by comparing treatment data to the negative control only. Data were then tested for normality using the Shapiro-Wilk test ($\alpha = 0.01$) and for homogeneity of variance using either the Bartlett ($\alpha = 0.01$) or Levene test ($\alpha =$ 0.01). Endpoints that met these assumptions were analyzed using ANOVA followed by the Dunnett Multiple Comparison test. Endpoints failing one or both of these assumptions were analyzed using the non-parametric Mann-Whitney U Two Sample test. Day 20 growth exhibited a suggestive, though non-linear, decreasing trend and was also analyzed using the Williams Multiple Comparison Test. Analyses were conducted using the reviewer-calculated time-weighted average bulk sediment concentrations, but were converted to the TWA OC-normalized sediment and TWA porewater concentrations. Significance was set at $\alpha = 0.05$, unless specified otherwise.

Endpoint	Methods	TWA Sediment (μg ai/kg)	TWA OC Sediment (µg ai/g OC)	TWA Porewater (µg ai/L)
Day 20 Survival	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Day 20 AFWD	Dunnett/Williams	NOAEL: 46 LOAEL: 96	NOAEL: 1.7 LOAEL: 3.3	NOAEL: 0.41 LOAEL: 0.69
Male Dev. Rate	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Female Dev. Rate	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Combined Dev. Rate	Mann-Whitney	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Male:Female	Mann-Whitney	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Time to Death	Mann-Whitney	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69

Endpoint	Methods	TWA Sediment (μg ai/kg)	TWA OC Sediment (µg ai/g OC)	TWA Porewater (μg ai/L)
Eggs/Emerged Fem.	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Eggs/Egg Case	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Eggs Cases/Fem.	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Percent Emergence	Dunnett	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69
Time to Oviposition	Mann-Whitney	NOAEL: 93 LOAEL: >93	NOAEL: 3.3 LOAEL: >3.3	NOAEL: 0.69 LOAEL: >0.69

13. <u>REVIEWER'S COMMENTS</u>:

The reviewer's conclusions were comparable to those reported by the study author, but were based on the time-weighted average bulk sediment, OC-normalized sediment, and porewater concentrations. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

The reviewer's analyses detected significant reductions in mean time to death, eggs per egg case, egg cases per emerged female, and female development rate. However, these reductions did not appear to be treatment-related, with the reductions always occurring at mid-level concentrations, and one or more higher treatment levels being statistically comparable to the negative control.

The number of eggs per egg mass averaged 776 and 742 for the negative and solvent control levels, respectively, which was slightly below the minimum standard validity criteria established by EPA Test Method 100.5 (of ≥800). The study author reported that the majority of the egg masses from the control groups were observed to be normal and the percent hatch was above acceptability criteria. It was further noted that the EPA test method that was originally used to generate the reproductive acceptability criteria included the use of both formulated and natural sediment, and that natural sediment may have additional nutrition sources that could lead to larger individual female adult midge and, therefore, larger egg masses. The study author concluded that as it is unclear of the validity of this acceptability criteria and percent hatch was

within the acceptable range, the control eggs per egg mass data is considered acceptable for risk assessment purposes.

In addition to mean-measured sediment concentrations, results were reported (by the study author) in terms of OC-normalized sediment concentrations. The organic carbon content of the formulated sediment was 2.8%.

Nominal	Mean-measured	OC-Normalized
Sediment	Sediment	Sediment
(µg ai/kg)	(µg ai/kg)	(µg ai/g OC)
6.3	5.2	0.19
13	12	0.43
25	22	0.79
50	45	1.6
100	89	3.2

The reviewer calculated the time-weighted average concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C TWA is the time-weighted average concentration,

C j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j

(e.g., t = 0 hours (test initiation), t = 24 hours, t = 296 hours)

Though the measured sediment concentrations were relatively stable over the duration of the exposure period (CV of <20% in all treatment groups), the measured porewater concentrations were highly variable (CV of 19.9-40.9%). For consistency, the reviewer calculated the sediment TWA concentrations, which were then corrected for the sediment organic carbon content (2.8%).

On Day 38, replicate A of the 12 μ g/kg treatment level was accidentally dropped by the laboratory technician during daily maintenance of the exposure and this replicate was excluded from water quality measurements and biological observations.

Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 160 to 320%. The study author reported that the variable results were most likely due to the large amount of sediment being dosed for these studies, and not having sufficient space to mix thoroughly in the standard mixing jars during the equilibration period. It was reported

that at the time the pretest results became available, a discussion with the Sponsor was initiated and it was agreed to add a mechanical mixing step prior to allocating the sediment to the test vessels. It was further reported that as analysis of samples during the in-life portion of the exposure yielded concentrations that were within the expected range, it was assumed that the sediment was thoroughly homogenized at the time of sediment allocation to the individual test replicates (day -1).

Sediment and pore water (exposure and QC) samples were analyzed using liquid chromatography with mass spectrometry (LC/MS/MS) and gas chromatography with mass selective detection (GC/MSD), respectively, based on methodology validated at Smithers Viscient. The sediment method validation study was conducted prior to study initiation and established an average recovery of $87.6 \pm 7.44\%$ from artificial sediment and $97.9 \pm 11.6\%$ from marine sediment. The aqueous matrix method validation was also conducted prior to study initiation and established an average recovery of $91.2 \pm 4.98\%$ from filtered seawater. It was reported that conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validations.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high Koc values) and regular renewal of the overlying water.

The dissolved organic carbon (DOC), total organic carbon (TOC), ammonia (as N), temperature and pH were measured in isolated pore water at each level on Days 0, 20, and 63. The DOC declined during the study from 110 to 130 mg/L on Day 0 to 32 to 50 mg/L by Day 63. The TOC also decreased from 140 to 170 mg/L on Day 0 to 58 to 71 mg/L by Day 63 (the majority of Day-20 samples were lost due to a software malfunction during analysis; however, it appeared from the remaining samples that concentrations were declining during the study). Ammonia levels declined from maximums of 3.6 to 4.3 mg/L (as N) on Day 0 to 0.23 to 1.2 mg/L by Day 63. The temperature and pH ranged from 21 to 23° and 6.4 to 6.9, respectively, during the study.

The experimental phase of the definitive test was performed from July 10 to September 11, 2013.

14. <u>REFERENCES</u>:

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